

Next Generation of Advanced Laser Fluorescence Technology for Characterization of Natural Aquatic Environments

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LONG-TERM GOALS

The project research addresses our long-term goal to develop an analytical suite of the Advanced Laser Fluorescence (ALF) methods and instruments to improve our capacity for characterization of aquatic environments. The ALF technique (Chekalyuk and Hafez, 2008) uniquely combines spectrally and temporally resolved measurements of the laser-stimulated emission (LSE) to provide assessments of key variables, including chlorophyll *a* (Chl *a*), chromophoric dissolved organic matter (CDOM), and phycobiliprotein-containing phytoplankton and cyanobacteria. The pump-during-probe measurements of variable fluorescence, F_v/F_m , yield assessments of phytoplankton photophysiological status. An extensive series of ALF measurements in diverse water types has demonstrated ALF utility as an integrated tool for aquatic research and observations. The ALF integration into the major oceanographic programs is currently in progress, including the California Current Ecosystem Long Term Ecological Research (CCE LTER, NSF) and California Cooperative Oceanic Fisheries Investigations (CalCOFI, NOAA).

OBJECTIVES

The specific goal of the project is to develop the next generation, commercial ALF sensors for oceanographic research, validation of satellite ocean color data, and environmental monitoring. The objectives are:

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1. To develop the Aquatic Laser Fluorescence Analyzer (ALFA) for laboratory and field applications, including discrete sample analysis and underway shipboard measurements.
2. To develop the ALF In Situ (ALFIS) fiber-probe sensor.
3. To integrate, test and deploy the ALFA and ALFIS sensors on the solar-powered AUVs and research cruises.
4. To initiate operational use of the new ALF instruments for ecological and biogeochemical measurements, and validation of ocean color remote sensing.

The project research addresses NOPP BAA 2009 subtopics: 2.1 Integration of ... in situ ... bio-optical sensors on nontraditional or novel sampling platforms; 2.2A Development of the next generation of ... bio-optical field sensors to further exploit current "ocean color" satellite data, and/or new observations from ocean color satellite retrievals; 2.2B Development of enhanced or new laboratory instrumentation for ecological or biogeochemical measurements in support of ocean color remote sensing.

APPROACH

The project work is conducted in close collaboration between Lamont-Doherty Earth Observatory (LDEO) of Columbia University as a lead organization, WET Labs, Inc., as an industrial partner, and Scripps Institution of Oceanography (SIO). The basic suite of optical and electronic modules and software was selected, designed and developed in Y1 and Y2 to integrate in the ALF sensors. The ALF analytical algorithms were refined via a series of laboratory and field measurements and integrated in the ALF software. The ALF sensors have been calibrated and validated at LDEO, tested at SIO and operationally deployed on a series of research cruises in collaboration with the CCE LTER and CalCOFI programs. In addition, it is planned to integrate and field-test the new instruments on solar-powered AUV particularly suitable for validation of ocean color data and long-term autonomous deployments. The work plan includes analysis of the ALF field data for assessment of the accuracy thresholds and uncertainties in satellite retrievals of Chl-a, CDOM and other relevant variables.

Key individuals: The **Principal Investigator**, Dr. **Alexander Chekalyuk**, is an expert in laser fluorescence, bio-environmental monitoring, and oceanographic research. He manages the project, leads the instrument development and modifications, plans and personally participates in the design and technological development, lab and field tests. Dr. **Andrew Barnard**, a project CoI, is a **WET Labs** Vice President of Research and Development. He manages all aspects of WET Labs work on engineering design and development in coordination with the LDEO team. Mr. **Casey Moore** (WET Labs President) is involved in the project as WET Labs Principal Engineer; he also provides oversight on the programmatic activities. Dr. **Mati Kahru**, a project CoI from **Scripps Institution of Oceanography (SIO)**, has a broad knowledge in biological oceanography and is a lead expert in ocean color satellite remote sensing. He collects and analyzes the relevant data from the available satellite sensors in support of the ALF field deployments. Mr. **John Baker**, the President and General Manager of **Falmouth Scientific, Inc. (FSI)**, personally represents a project subcontractor, FSI, responsible for integration and deployment of the ALFIS sensor on the solar powered SUVII AUV. Dr. **Andrew Juhl**, Doherty Associate Research Scientist, is a biological oceanographer who assists this project by acquiring and maintaining phytoplankton cultures necessary for calibration of the instruments.

WORK COMPLETED

The **Y2 project work** was conducted in a series of Tasks outlined in the Work Plan of the original proposal:

- Task 1. Refinement of ALF analytical algorithms
- Task 2. Development of Aquatic Laser Fluorescence Analyzer (ALFA)
- Task 3. ALFA field tests and operational deployments

Task 4. Development of ALF In Situ (ALFIS) sensor

Task 6. Validation of ocean color products using ALF field measurements

The ALF analytical algorithms were further refined, including improving fluorescence assessment of chlorophyll concentration, and extension of the ALF analytical capabilities for assessment of phytoplankton biomass and community composition. Several prototypes of the commercial benchtop ALFA instrument were designed, built, calibrated and successfully tested in laboratory and field conditions, including 3 research cruises in the Pacific Ocean (Figs. 1-3). The ALFA operational and analytical software was developed, field-tested, and integrated with the shipboard data acquisition system. The design solutions for the ALFIS sensor were analyzed, including a new compact single-laser optical configuration with a new type of laser. A brief description of the Y2 project accomplishments can be found below in the “Results” section of the Y2 project report.

RESULTS

Task 1. Refinement of ALF analytical algorithms. Photo-physiological variability of *in vivo* chlorophyll fluorescence (CF) per unit of chlorophyll concentration (CC) was analyzed to improve the accuracy of CC assessments. ALF field measurements of CF and photosystem II (PSII) photochemical yield (PY) were analyzed vs. high-performance liquid chromatography (HPLC) CC retrievals. A four-step measurement protocol was developed and successfully tested. It provides the accuracy of CC fluorescence measurements comparable to the accuracy of commonly accepted preparatory methods, such as HPLC (dark dots in Fig. 4; $R^2 = 0.90$). The four-step protocol includes (1) isolation from ambient light, (2) PSII saturating excitation, (3) optimized phytoplankton exposure to excitation, and (4) phytoplankton dark adaptation. For *in situ* or flow-through measurements, concurrent PY measurements can be used to adjust for CF non-photochemical quenching (NPQ) and improve the accuracy of CC fluorescence assessments. Field evaluation has shown the NPQ -invariance of CF/PY and $CF(PY^{-1}-1)$ parameters and their high correlation with HPLC CC retrievals ($R^2 = 0.93$ in Fig. 4B), while the NPQ -affected CF measurements correlated poorly with CC ($R^2 = -0.22$ in Fig. 4A). This significant methodological improvement was described in the manuscript submitted for peer review publication in Optics Express (Chekalyuk and Hafez, 2011).

The ALF fluorescence measurements in the offshore waters in the California Current ecosystem have revealed quite different patterns. Strong, but non-linear regression relationship was found between CF and CC (Fig. 5A; $R^2=0.95$). This suggests that the conversion of CF measurement into CC units may need to be adjusted with regard to the specifics of the aquatic environment (e.g., “estuarine” vs. “offshore”). During Y3 of the project research, we plan to explore a potential of the ALF spectral recognition of the water types to select the appropriate $CF \Rightarrow CC$ conversion procedure. The high correlation between CF and carbon biomass of autotrophic phytoplankton ($R^2=0.79$; Fig. 5C) indicates potential for reasonably accurate fluorescence assessment of phytoplankton carbon biomass. This is another important methodological result that may lead to further extension of the analytical capabilities of fluorescence analysis. For example, Fig. 6 presents 2D spatial distributions of Chl a concentration and carbon biomass in the euphotic layer across the frontal zone calculated using ALF fluorescence measurements. The high linear correlation was also found between the ALF measurements of phycoerythrin fluorescence (F_{PE12}) of phototrophic cyanobacteria *Synechococcus* in the subsurface waters and their carbon biomass, SYN ($R^2=0.95$; Fig. 5D). This can be used for fluorescence assessment of phytoplankton structural composition. These results are described in a manuscript to be submitted in Oct. 2011 for peer review publication in the Journal of Plankton Research (Chekalyuk et al. 2011).

Task 2 Development of Aquatic Laser Fluorescence Analyzer (ALFA). The ALFA instrument development is being conducted in partnership between LDEO and WET Labs. The ALF system design, component selection and measurement protocols has been critically analyzed to reduce the cost, size, weight, and power consumption of the ALFA instrument, and to improve the overall system performance and robustness. The LDEO and WET Labs teams have held a series of project meetings to evaluate the overall architecture of the ALF prototypes and discuss the design options for the ALFA instrument (Fig. 1). The key ALF components have been reviewed for potential improvements in reliability, manufacturability, and supportability. The first ALFA prototype (Fig. 7) was built by WET Labs in Dec. 2010, and tested and evaluated in laboratory conditions in Jan-March 2011. Two additional ALFA prototypes implementing the improved design were built during this period. The basic ALFA configuration incorporates a new lightweight modular rail optical mounting system and includes the 405 nm and 532 nm lasers, CCD spectrometer, and PDP sensor of variable fluorescence. An external ToughBook laptop computer is used for the instrument control and data acquisition. The ALFA operational software and user interface have been significantly reworked and modified to allow the instrument use by researchers and technicians without special training, simplify the user interface and data saving format.

Task 3. ALFA field tests and operational deployments. In Apr. – Aug. 2011, two ALFA prototypes were tested in the field by the LDEO/SIO scientists on two CalCOFI and one CCE LTER cruises (Fig.3). The ALFA instruments were integrated with the shipboard sampling system of the R/V “Melville”, that provides measurements of seasurface temperature, salinity, oxygen and Chl *a* fluorescence. This instrument configuration was used for high-resolution underway measurements of the key bio-environmental variables (for example, Fig. 8) and discrete sample analysis that provided valuable data for the CCE LTER research program (NSF) and CalCOFI long-term monitoring (NOAA). The ALFA measurements were used for selecting the sampling sites in the complex and highly variable areas of strong gradients in California Current ecosystem (Fig. 8). The field tests have been used to further refine the ALFA design. In particular, the water sampling system is been currently modified to increase the flow rate and minimize the sample exposure to the excitation.

Task 4. Development of ALF In Situ (ALFIS) sensor. The ALFIS sensor will implement the core solutions and analytical protocols of the ALF technology (Chekalyuk and Hafez, 2008), but its design, selection of components and measurement protocols need to be modified with regard to specifics of *in situ* measurements. The ALFIS design solutions are currently been analyzed and evaluated by the project team. We have already identified a new type of laser for conducting both spectrally and temporally resolved fluorescence measurements. This single-laser optical configuration will allow us to simplify instrument design, and reduce its size and power consumption. This laser has been recently custom-built and breadboard-tested by the LDEO project team (Fig. 9) to evaluate feasibility of conducting the ALF measurements. Both spectrally and temporally resolved measurements have shown to be feasible. In addition, better spectral separation between the overlapped spectral components was achieved to improved fluorescence retrievals in low-productive offshore waters. After the completing the ongoing breadboard tests and evaluation, the project team will hold a meeting to evaluate the options for ALFIS instrument design and development. Based on the initial successful laser tests, it seems realistic to develop the ALFIS instrument in the timeframe planned in the original proposal. We plan to conduct the initial ALFIS field tests at the SIO pier in May-June 2012 and deploy the instrument on the CCE LTER cruise in July 2012. Two prospective towed platforms, the Moving Vehicle Profiler (MVP) and SeaSoar (Fig. 10), are considered along with the CTD rosette for deploying the ALFIS. Our subcontractors, Falmouth Scientific, Inc., will assist on the ALFIS deployment on a solar-powered SAUV.

Task 6. Validation of ocean color products using ALF field measurements. The LDEO and SIO project teams have continued their work on the comparative analysis of the existing ALF underway data vs. satellite retrievals. The ALF horizontal transect measurements have been shown to be useful for assessing the accuracy and uncertainties in satellite ocean color retrievals. ALF technique is uniquely positioned vs. ‘regular’ fluorometry as it yields more accurate assessment of pigment concentration and better addresses the different footprints of the satellite and typical *in situ* sensors. We have continued working on the methodology of inter-comparisons between the ALF and satellite data, focusing on the Chl *a* concentration, the most important product of the satellite ocean color measurements. The data sets acquired in the contrast areas of California Current during CCE LTER cruises have been analyzed.

An example of the ALF synoptic-scale (311 km) validation of the satellite retrievals is presented in Fig. 11. The 15-hour high-resolution ALF measurements (9588 measurements, 1 sampling every 32 m) in the California Current on October 14-15, 2008 during the CCE LTER cruise. The ship track is displayed with a black line in the sea-surface temperature (SST) and Chl *a* maps (panels A and B; MODIS-Aqua data of Oct. 16). The transect distributions of MODIS retrievals and most relevant ALF variables are presented in panel C along with the HPLC measurements of total Chl *a* (red dots). As evident from panel C, all three Chl *a* data sets have shown remarkable correlation over the most offshore, west portion of the transect, but the MODIS-Aqua algorithm did not resolve the sharp, almost 2-fold drop in Chl *a* detected by the ALF at the temperature front. This drop was accompanied by the significant changes in other variables measured by the ALF (e.g., the sharp rise in CDOM, F_v/F_m (an index of phytoplankton photochemical efficiency), and PE1 phycoerythrin fluorescence (indicative of blue-water cyanobacteria)). By contrast, in the east portion of the transect the MODIS-Aqua Chl *a* retrievals significantly, 20-30%, underestimated both ALF and HPLC Chl *a* measurements. This example shows that ALF measurements conducted from the moving platform (research vessel or AUV) can be used as a cost-effective solution for validation of the satellite ocean color algorithms. The multivariable ALF data can be potentially help in understanding the origin of relatively poor performance of the ocean color algorithms at some locations. We are currently working on a manuscript summarizing the initial results of the ALF satellite validation, and we will continue this study in Y3 of the project research. After refining the approach with the existing ALF data, the new validation technique will be applied to the new measurements conducted during the field deployments of the ALFA and ALFIS sensors.

IMPACT and APPLICATIONS

The project research addresses NOPP BAA subtopics: 2.1 Integration of ... *in situ* ... bio-optical sensors on nontraditional or novel sampling platforms; 2.2A Development of the next generation of ... bio-optical field sensors to further exploit current "ocean color" satellite data, and/or new observations from ocean color satellite retrievals; 2.2B Development of enhanced or new laboratory instrumentation for ecological or biogeochemical measurements in support of ocean color remote sensing. The project builds upon state-of-the-art scientific and technological advances to provide the scientific community and government agencies new means for research, observations and environmental monitoring in diverse aquatic environments. The ALFA sensor will provide high-resolution shipboard underway flow-through measurements and sample analyses over a range of spatial and temporal scales. The ALFIS sensor will be used for deployments from a variety of platforms, including autonomous unmanned vehicles, automatic gliders, vertical and drift profilers, buoys, and moorings, thus contributing to development of the Ocean Observing Systems and other emerging initiatives.

Economic Development

WET Labs is a company that works at the forefront of technology and provides state-of-the-art oceanographic sensors and platforms. The new ALFA and ALFIS sensors will create conceptually new line of products enabling new unique measurement and observational capabilities. The extensive series of field deployments of the prototype ALF instrument has already attracted significant interest to the new technology in the research community and government agencies. The completion of this NOPP project and commercialization of the ALF technology will create new jobs and promote local and regional economic development.

Quality of Life

The ALFA and ALFIS sensors will provide measurements of the key bio-environmental variables, including chlorophyll-a biomass, phycobiliprotein pigments, chromophoric dissolved organic matter, as well as basic characterization of the structure and photo-physiological status of the mixed populations of phytoplankton and cyanobacteria in natural waters. The operational use of the new technology in oceanographic research and environmental monitoring will provide new observations and data to address the emerging problems of particular importance in coastal, estuarine and inland aquatic environments, such as ecosystem health and biodiversity, harmful algal blooms, eutrophication, pollution, fisheries, coastal management, etc., which may ultimately help to improve the quality of life.

Science Education and Communication

The potential future impact of developing the next generation on the ALF technology will include introduction of the the new scientific data and developments incorporated in the ALF measurement and analytical algorithms to the engineering community and public sector. The initial results of our NOPP project have been reported at the XX Ocean Optics meeting (Anchorage, Alaska). Several talks and presentations on the ALF technology, applications and commercialization and are planned at the Special Session # 127 on Pigment Fluorescence aorginzed by the Project PI, Dr. Chekalyuk, at the AGU Ocean Sciences meeting in Feb. 2012 at Salt Lake city (Utah). These meetings are attended by signifcant amount of undergraduate and graduate students and researchers. We will continue informing the scientific community and public about our NOPP project results and accomplishments via publications, presentations and internet web sites.

TRANSITIONS

Quality of Life

The California Cooperative Oceanic Fisheries Investigations (CalCOFI, NOAA) program has already deployed the prototype ALF instruments in collaboration with LDEO of Columbia University on several seasonal CalCOFI cruises in the Pacific coastal zone of California and California current to measure spatial patterns of the key bio-environmental variables over large aquatic areas. They will collaborate with our NOPP project team on the field tests and deployments on the new technology to ultimately incorporate it into the long-term CalCOFI monitoring program. The successful operational incorporation of the ALF technology into the CalCOFI program may provide new data to improve California coastal fisheries and resource management.

Science Education and Communication

The California Current Ecosystem Long Term Ecological Research (CCE LTER, NSF) program has already deployed the prototype ALF instruments on several resaerch cruises in the Pacific coastal zone

of California and California current. The program has been recently extended by NSF for additional 6 years with specific emphasis on the role of meso-scale physical and biological structures on the California Current Ecosystem. They have already ordered a prototype of the ALFA system that they will use on their research cruises and during the laboratory studies. The instrument will be built in December 2010 and transferred to Scripps Institute of Oceanography, the lead organization of the CCE LTER program. Our NOPP project team will train the CCE LTER personnel and participate in the field deployments. The acquired data will be used to provide important feedbacks to further improve the new generation of the ALF technology.

RELATED PROJECTS

“Collaborative Research: Advanced Laser Fluorometer (ALF) for in vivo Characterization of Phytoplankton Pigments, Physiology and Community Structure”. NSF Ocean Technology and Interdisciplinary Research Program, Award # OCE-07-24561, May 2007- April 2010. Budget 462K, CoI: B. G. Mitchell (Scripps Institute of Oceanography, UCSD); Status: completed in 2011.

“RAPID: Rapid Assessment of Extent and Photophysiological Effects of the Deepwater Horizon Oil Spill”; NSF OCE; Award # OCE-1048482; June 2010 – July 2011; Budget: \$199,972; CoIs: A. Subramaniam and A. Thurnherr (LDEO of Columbia University, NY); Status: in progress

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Chekalyuk, A.M. and M. Hafez. Advanced laser fluorometry of natural aquatic environments. *Limnol. Oceanogr. Methods* 2008, 6:591-609

A. Chekalyuk and M. Hafez, "Photo-physiological variability in phytoplankton chlorophyll fluorescence and assessment of chlorophyll concentration," *Optics Express*. **19**(23), 22643–22658 (2011)

Chekalyuk, A.M., M.R. Landry, R. Goericke, A.G. Taylor, and M. Hafez. Laser fluorescence analysis of phytoplankton across a frontal zone in the California Current ecosystem. *J. Plankton Res.* 2011 (submitted Nov. 4, 2011; in peer review)

PUBLICATIONS

A. Chekalyuk and M. Hafez, "Photo-physiological variability in phytoplankton chlorophyll fluorescence and assessment of chlorophyll concentration," *Optics Express*. **19**(23), 22643–22658 (2011)

PATENTS

“Spectral and Temporal Laser Fluorescence Analysis such as for Natural Aquatic Environments”, Inventor/Applicant: Alexander Chekalyuk; EFS ID: 8964632; International Application Number: PCT/US10/58891; Status: pending; this patent application on the ALF technology and methods was filed with the Science and Technology Ventures office of Columbia University in Nov. 2010.



Fig. 1. An ALF prototypes developed by LDEO team have been critically analyzed when designing the ALFA instrument (Mr. Derr and Dr. Zaneveld at the WET Labs team meeting, Oct. 2010).



Fig. 2. Laboratory tests and calibration of the ALFA instruments by the LDEO/Wet Labs project team, March 2011.



Fig. 3. Field deployment and tests of the ALFA instruments by the LDEO project team in collaboration with Scripps Institution of Oceanography on the NSF-sponsored California Current Ecosystem Long Term Ecological Research (CCE LTER) cruise, June-July 2011.

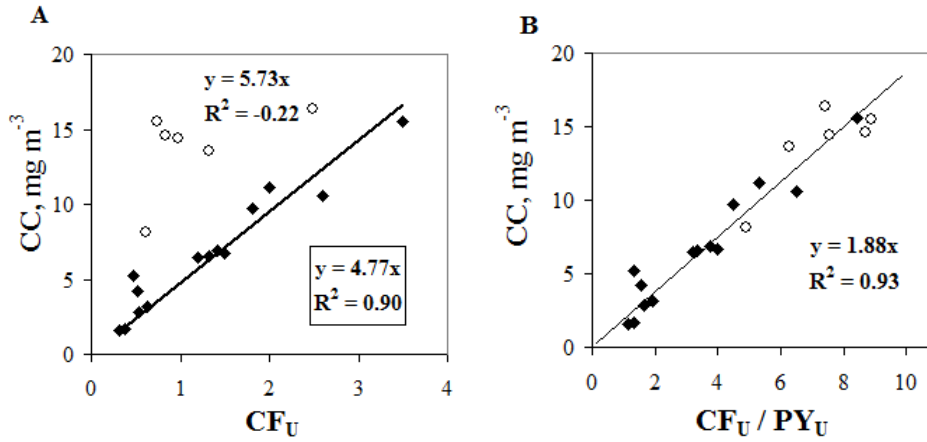


Fig. 4. (A) Correlation between measurements of Chl concentration (CC) in water samples and underway Chl fluorescence measurements at the sampling locations (CF_U). Black dots and empty circles represent the data from the dark-time and morning portions of the transect, respectively. The framed and unframed regression equations are calculated for the dark-time and entire data sets, respectively. (B): Correlations between CC in water samples and fluorescence parameter CF/PY calculated from Chl fluorescence (CF) and PSII photochemical yield (PY) underway flow-through measurements (the same data set as in panel A).

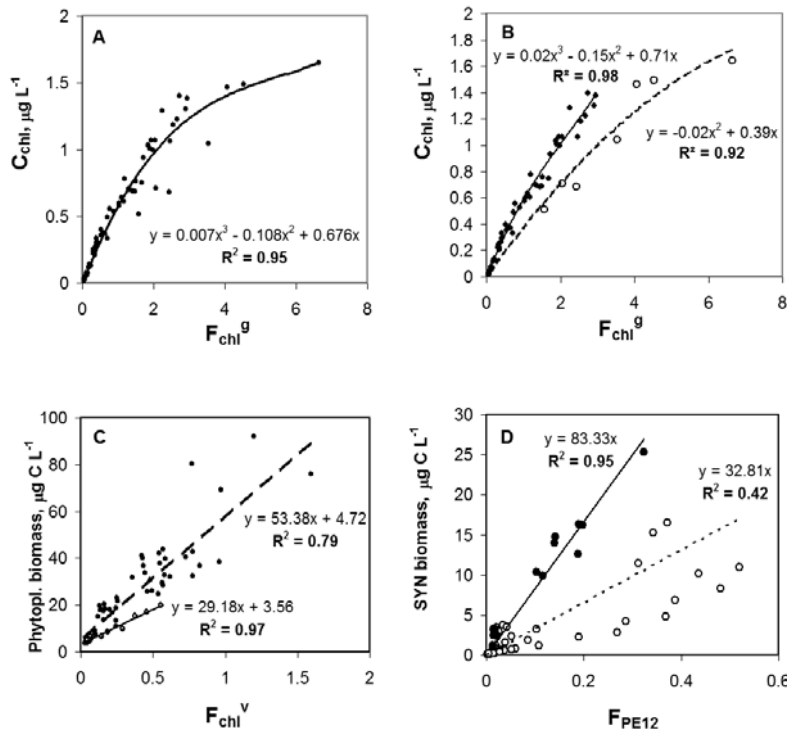


Fig. 5. (A): Correlation between chlorophyll a (Chl a) concentration (C_{chl}) and Chl a fluorescence (F_{chl}) measured with ALF instrument. (B): Same data as in panel A; a subset of samples from the diatom-dominated Chl a maximum at the front is displayed with empty circles. (C): dashed regression line – correlation between autotrophic carbon biomass (AC) and Chl a fluorescence (F_{chl}) measured with ALF instrument; solid regression line – correlation for a subset of samples collected between 40 m and 80 m at Stns. 1-3. (D): dashed regression line - correlation between phycoerythrin fluorescence of *Synechococcus* (F_{PE12}) and their carbon biomass (SYN); solid regression line - correlation for a subset of the samples (filled dots) collected in the subsurface layer above the Chl a maximum.

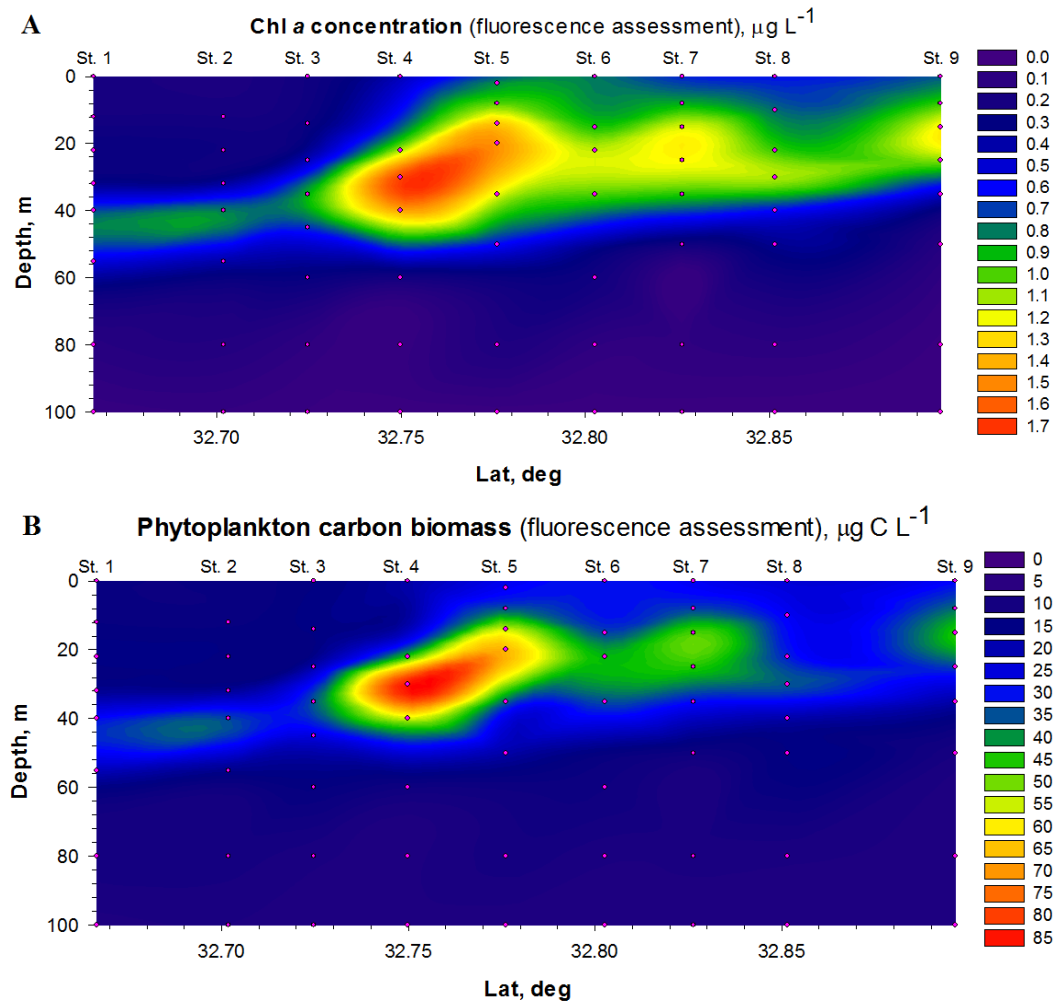


Fig. 6. Distributions of Chl a concentration (A) and carbon biomass of autotrophic phytoplankton (B) in the euphotic layer across the frontal zone calculated using Chl a fluorescence measurements of discrete water samples.



Fig.7. A prototype of commercial Aquatic Laser Fluorescence Analyzer (ALFA) developed by the LDEO/WET Labs project team.

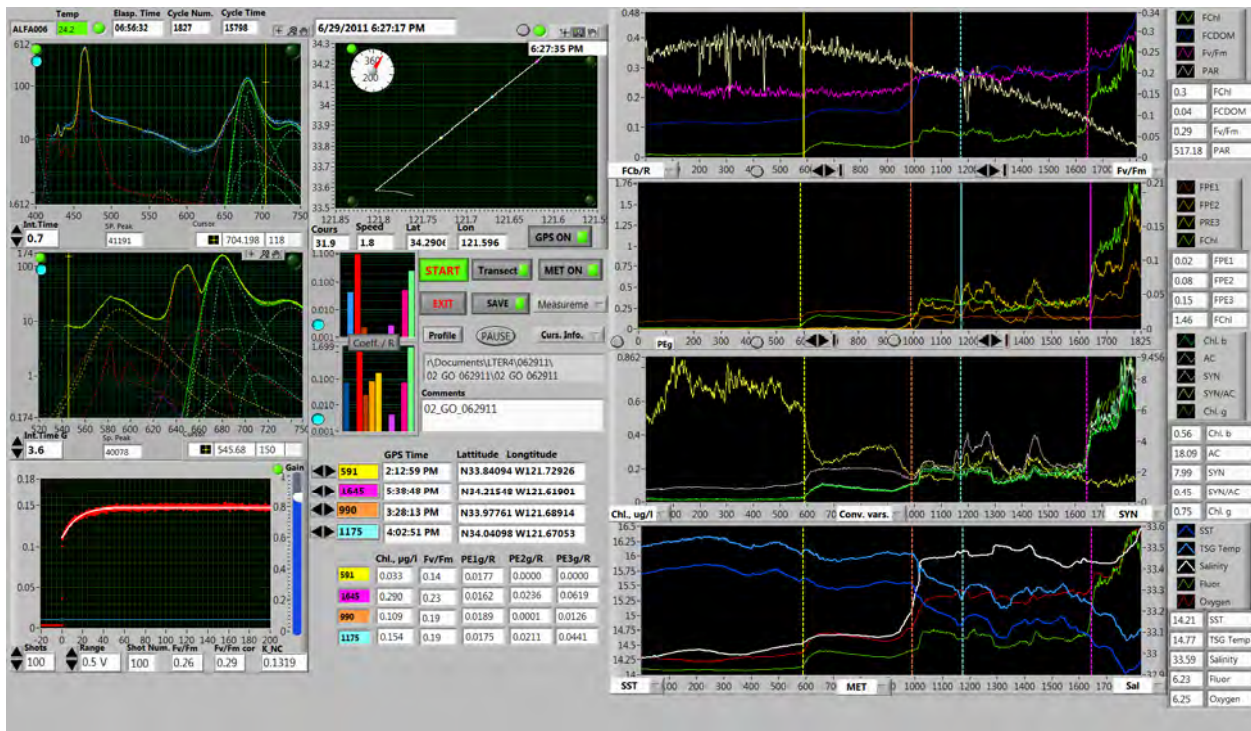
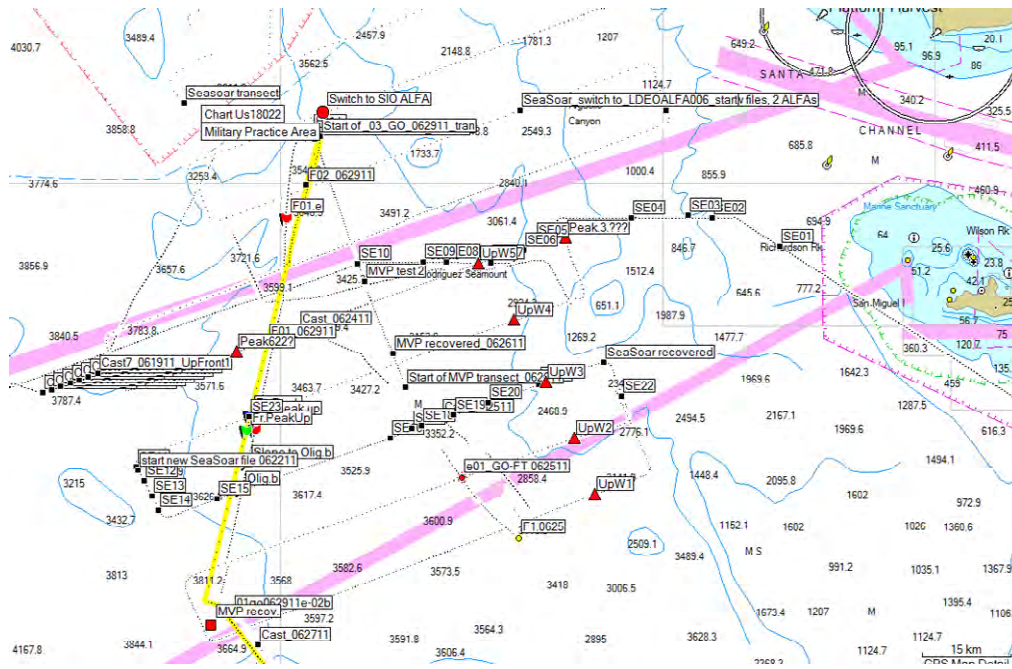


Fig. 8. A transect map and a screenshot of the ALFA operational software taken during the underway frontal study with the ALFA instrument in the California Current System, CCE LTER cruise, June 2011. Right panels display spatial distributions of Chl a (green), CDOM (dark-blue, upper panel), Fv/Fm (magenta), PAR (beige), PBP pigments (yellow-orange), temperature (blue), salinity (white), and oxygen (red).

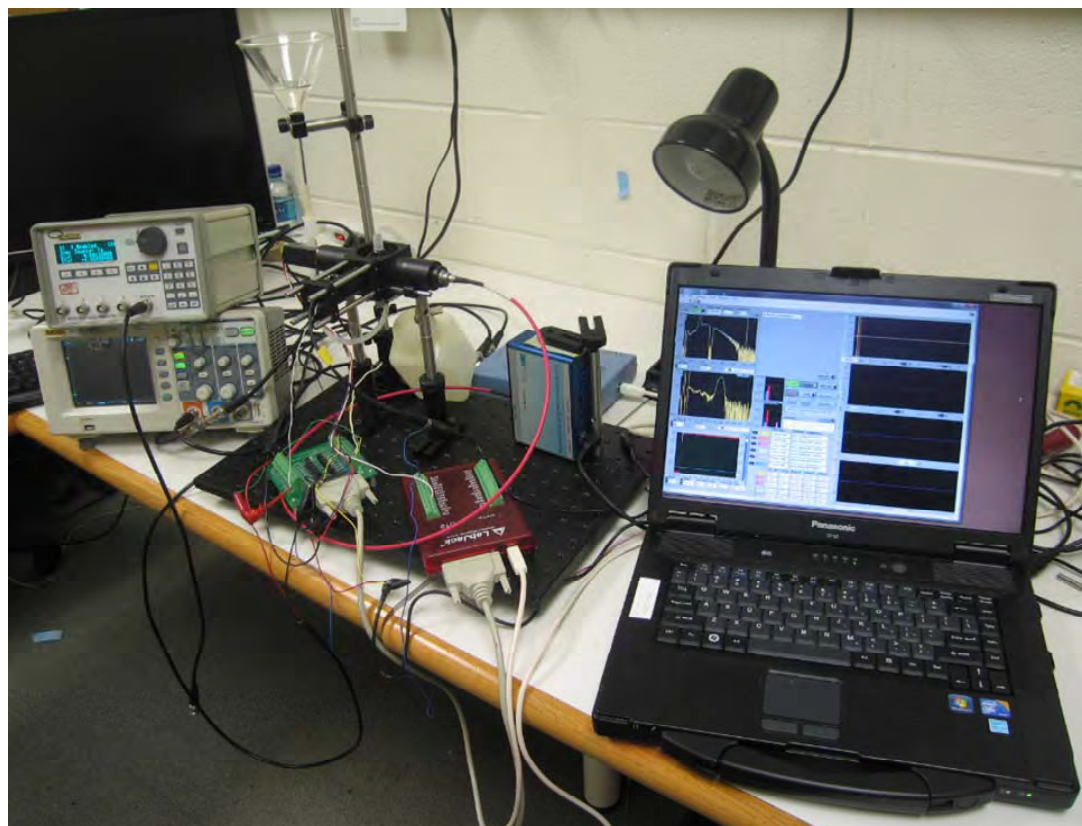


Fig.9. A breadboard setup in the LDEO optical laboratory to develop the ALF In Situ (ALFIS) optical configuration. It is currently used to test a new type of laser for a compact single-laser ALFIS optical design.

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Fig. 10 The Moving Vehicle Profiler (MVP, upper photo) and the SeaSoar (lower photo) towed vehicles are considered as prospective platforms for deployments of the next generation, ALF In Situ instrument to be built during Y3 NOPP project research.

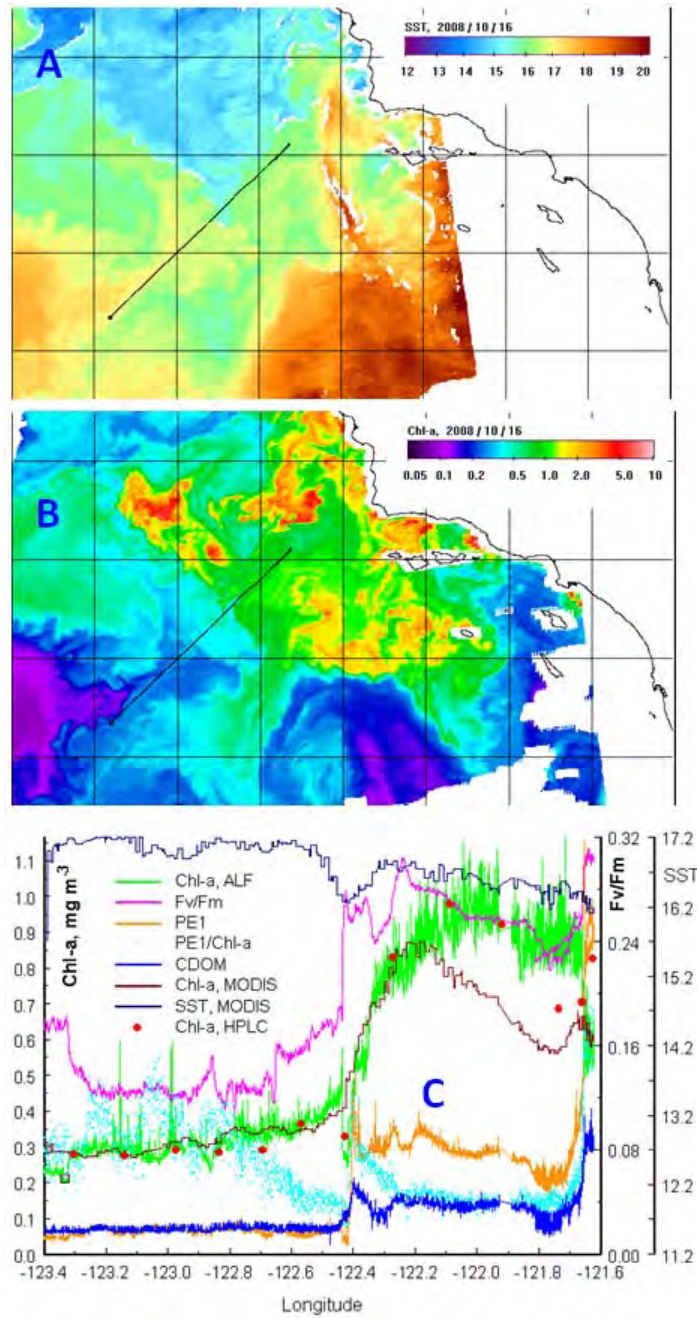


Fig. 11. Matching MODIS Aqua SST (A, C) and Chl-a (B, C) data with ALF multivariable shipboard underway (C) and HPLC Chl a (C) measurements. Ship track: black line in (A) and (B). California Current, CCE LTER cruise, Oct. 14-15, 2008. The ALF high-resolution (~30 m) measurements can be used for validation of the satellite retrievals, assessment of sources uncertainties, and development of the new ocean color products (e.g. PE biomass, phytoplankton functional types, etc.).